8.

Sex Determination in *Platypoecilus maculatus*. I. Differentiation of the Gonads in Members of All-male Broods.[1]

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(Plates I-IV; Text-figures 1-3).

In the course of studying the sex-linked inheritance of macromelanophores in various strains of the viviparous platyfish, *Platypoecilus maculatus*, two females were found producing large numbers of offspring all of which were male. A genetic mechanism for sex determination was known which could explain the results obtained. For example, Gordon (1946) indicated that when a male platyfish of domesticated stocks is mated with a female of wild-caught stocks from Mexico, all the young are male. It was explained that domesticated stocks have a type of genetic mechanism for sex determination in which the female is WY and the male is YY, while in the wild stocks from Mexico the female is XX and the male is XY. Thus when an XX female is mated with a YY male, all the young are XY, and male.

If the suggested chromosomal mechanism for sex determination is valid when applied to the male-producing females, it was thought that a histological study of the developing gonads of the offspring should reveal the presence of testicular anlagen in the extremely young and definitive testes in the older members of the all-male broods. This paper reports the histological results obtained.

As the studies progressed it was found that the material was suitable to show the relationship of the various stages in the development of the testes to corresponding stages in the transformation of the male's anal fin into the highly complex gonopodium, the fin which serves to transfer the spermatophores from the male to the genital aperture of the female. In the platyfish fertilization is internal and the females produce about 40 living young at intervals of about 28 days.

There was an opportunity also to study the relationship of the stage of development of the gonad to the age, size and genetic constitution of the fishes.

MATERIAL AND METHOD.

A female member (30^{8}-5) of an inbred (eighth generation) wild population of *Platypoecilus maculatus* originally from the Rio Jamapa, Veracruz, Mexico, was mated to the so-called "salt-and-pepper" male platyfish (BH-14) of a commercial stock obtained from the Everglades Aquatic Nurseries of Tampa, Florida.

The wild Rio Jamapa platyfish of stock 30 is characterized by being homozygous for the dominant striped gene *Sr*. This gene controls macromelanophores which form a series of faint but distinct rows of large black pigment cells on the sides.

The commercial "salt-and-pepper" platyfish is heavily spotted with macromelanophores. Ordinarily this stock carries the dominant gene *Sp* either in a homozygous or heterozygous state. Occasionally a member of this commercial stock also carries the dominant *N* gene, for macromelanophores arranged in a black-banded pattern. The male used, BH-14, phenotypically appeared to be *Sp*+, but actually was *SpN*, according to the results observed, Gordon (1951). The sex-linked genes, *Sr*, *Sp* and *N*, are members of an allelic series, according to Gordon (1948).

The genetic sex-determining mechanism of the Rio Jamapa platyfish was known to be XX female, XY male, from the work of Gordon (1947). The genetic analysis of the "salt-and-pepper" platyfish stock was in progress. It is now quite clear that this stock has the "domesticated" type of sex determination; that is, the WY female and YY male. This type, WY-YY, has recently been discovered in a natural population of *P. maculatus* from the Belize River in British Honduras, according to the announcement of Gordon (1950a).

The mating between platyfish of differing stocks may be expressed as follows:

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[3] This paper is based upon a thesis presented to the Graduate School of Arts and Sciences of New York University by the first author, in partial fulfillment for the degree of Master of Science.

P_1

Rio Jamapa Female (30^8-5)	Salt-and-Pepper Male (BH-14)
Striped-sided (X)*Sr*/(X)*Sr*	Spotted, Black-banded (Y)*Sp*/(Y)*N*

F_1 (Pedigree No. 263)

(X)*Sr*/(Y)*Sp*:
105 males, Spotted ("salt-and-pepper")

(X)*Sr*/(Y)*N*:
78 males, Black-banded

Every one of the 183 F_1 platyfish in the brood reared to maturity by routine methods of laboratory maintenance was a male. Female 30^8-5 continued to produce young and 40 of them (20 with spotted patterns and 20 with the black-banded patterns) were selected at varying ages and fixed in Bouin's fluid for histological study of their gonads. In addition female 30^8-5, when gravid again, at a later period, was sacrificed and 38 embryos in late stages of development were recovered from its ovary. All the embryos were examined carefully and were found normal; 13 of them were fixed, sectioned and studied histologically.

The genetic constitution of the second male-producing female, 239-1, can not be stated with precision, owing to the fact that it was obtained from a long series of matings involving both wild and domesticated stocks. This is also true of the male, 205-11, with which it was mated. In checking their genetic history it is probable that the female parent was XX and the male YY. Together they produced platyfish of brood No. 260, all 41 members of which were males. Eighteen of them were fixed in Bouin's fluid for histological study of their gonads.

After fixation, all the fishes were decalcified, dehydrated in dioxane, imbedded in paraffin and sectioned serially at eight microns. The sections were variously stained with Heidenhain's iron hematoxylin, Harris' alum hematoxylin, eosin and Heidenhain's modification of Masson's trichrome stain.

Before sectioning the fish, the condition of their anal fin was recorded. The standard length of each specimen was measured from the tip of the snout to the end of the caudal peduncle. The depth of the body, from the first ray of the dorsal fin to the first ray of the anal fin, was also measured, after which the body index was determined by dividing the value of the standard length by the value of the depth of the body. These measurements were recorded in tabular form, Tables 3 and 4. After sectioning, the stage of gonadal development of each animal was determined. These details are also included in Tables 3 and 4.

The culture methods used in the care and breeding of the platyfish are described in detail by Gordon (1950b).

The Development of the Gonad.

Introduction.

Wolf (1931) indicated that sexual differentiation in the platyfish may be detected histologically in embryos 6.0 to 6.5 mm. in length. Embryos having two hundred or more germ cells in the gonad are likely to be female; those having one hundred or less are probably male. He pointed out that a more reliable set of criteria is available for distinguishing the two sexes in slightly older, just born platyfish.

In 6 to 7 mm. early postnatal female platyfish, the germ cells, which have multiplied and enlarged to two or three times their original size, are distributed throughout the body of the primitive gonad. The stroma cells that surround the developing oögonia initiate the formation of the follicles.

In comparable 6 to 7 mm. postnatal male fish, the germ cells are distributed at the periphery of the gonad primordia; the center of the testis is occupied by stroma cells. In slightly older fishes the centrally located stroma cells cluster together to form the beginnings of the sperm duct.

These histological details of the differentiating gonads were utilized, in part, in the determination of the sex of the fishes under discussion.

The Undifferentiated Gonad.

Embryos.

Thirteen embryos in their fourteenth day of intra-ovarian development were studied.

They have small, widely separated gonad primordia consisting of undifferentiated mesodermal cells and primordial germ cells which are distributed throughout the gonad rudiment. The germ cells are oval and measure 8 to 13 microns (Pl. I, Fig. 1). Their cellular membranes are distinct and the cytoplasm is homogeneous except for the presence of occasional fine basophilia. Their nuclei at rest, which measure 5 to 9 microns, are almost spherical and their membranes stain deeply with hematoxylin. The chromatin granules are distributed throughout the nucleus but are more concentrated near the periphery. Each nucleus usually has one nucleolus, rarely two.

The mesodermal cells or stroma cells have no distinct cellular membranes. Their nuclei are characterized by being elliptical, and small, 3 to 4 microns, and each contains an irregular and coarse reticulum.

The gonad primordia are surrounded by peritoneal cells which are fusiform, have distinct cellular membranes and contain elliptical nuclei which are similar in size and structure to those of the stroma cells.

Early Postnatal Fish.

Six platyfish at birth, measuring 5.6 to 6.0 mm., were studied.

They have paired gonads which are larger and closer together than the two gonadal primordia of embryos. The primordial germ cells are found throughout the undifferentiated gonad.

The Development of the Testis.

Stage 1.

Nine platyfish, measuring 8.7 to 12.9 mm., were studied.

They have paired gonads which lie close together and are surrounded by a thin, simple, squamous, peritoneal membrane. The germ cells appear singly or in small groups at the periphery (cortex) of the gonad which, for the most part, is composed of stroma cells (Pl. I, Fig. 2). As indicated by Wolf, this peripheral distribution of the germ cells in platyfish of this size is indicative of a testis.

Stage 2. *Spermatogonial Acini.*

Nine platyfish, measuring 9.5 to 21.0 mm., were found in this stage of gonadal development.

They have paired gonads which have begun to fuse. An increasing number of germ cells are distributed about the periphery of the gonad in a discontinuous layer. There they form small groups or acini. Each acinus contains up to eight germ cells and these cells may now be referred to as spermatogonia (Pl. I, Fig. 3). The spermatogonial cells are approximately 5 to 6 microns in diameter and their nuclei are 3 to 4 microns. These cells, except for size, are similar to those of the primordial germ cells.

Some of the stroma cells, which are the major components of the developing testes, are grouped to produce a large sperm duct in each testis (Pl. IV, Fig. 11). The two ducts are fused posteriorly.

Stage 3. *Numerous Spermatogonial Acini.*

Ten platyfish, measuring 10.5 to 21.0 mm., were studied and found to be further advanced in their gonadal development.

The gonads are still only partially fused. A larger number of spermatogonial cells and acini form a thick cortex at the periphery of the gland (Pl. I, Fig. 4).

The proliferated stroma cells form a series of primary sperm ducts and a number of smaller branches or tubules. When viewed in cross-section these arborescent structures resemble a series of rings (Pl. I, Fig. 4).

Stage 4. *Primary Spermatocytes.*

Four platyfish, measuring 13.5 to 18.7 mm., were found to be in the fourth stage.

They have gonads in all parts of which are found a large number of spermatogonial acini, and an increasing number of acini that contain primary spermatocytes (Pl. II, Fig. 5). The interkinetic nuclei of the primary spermatocytes are spherical, measure approximately 3.5 microns, and each contains a coarse reticulum with several large chromocenters. The cellular membranes of the primary spermatocytes are not sharply defined.

The sperm ducts are larger and their branching tubules are more numerous. Some of them may extend almost up to the periphery of the testis.

Stage 5. *Secondary Spermatocytes.*

Nine platyfish, measuring 12.0 to 29.0 mm., were in this stage of development.

They have gonads which have completely fused, although the line of fusion is clearly visible. The acini are very numerous. Some of them contain spermatogonial cells, others contain primary spermatocytes, and some, secondary spermatocytes. The germ cells within a given acinus are all at the same spermatogenic stage. The primary and secondary spermatocytes are similar in appearance. There are twice as many secondary as primary spermatocytes in each acinus and their nuclei are half as large, about 2 microns. The acini which contain the secondary spermatocytes lie nearer the center of the testis than do the other types of acini. These acini develop in a lineal series, or cord, which is characteristic of this stage (Pl. II, Figs. 6 & 7).

The acini are encapsulated by a thin membrane of differentiated stroma cells. These cells are slender and elongate; their large, bulging nuclei are elliptical, 6 to 8 microns in diameter, and each contains a nucleolus.

Stage 6. *Spermatophores.*

Eleven young adults, measuring 15.1 to 26.8 mm., had large fused testes.

The testis is a single organ but its bipartite origin is clearly in evidence, for the two parts are unequal in size, the left lobe being larger than the right. It occupies a considerable area of the visceral cavity. The testis lies medially and is suspended beneath the swim bladder by a short mesorchium. It is covered by a thin, unpigmented peritoneal membrane.

The secondary spermatocytes within their acini first transform into spermatids and these, in turn, transform into spermatozoa. The heads of the spermatozoa, which measure 0.8 by 3.0 microns, are arranged peripherally within each acinus which now becomes a spermatophore (Pl. III, Fig. 8). The latter are 37 to 54 microns in diameter. The spermatophores within the germinal portion of the testes are surrounded by Sertoli cells (Pl. III, Fig. 9) which are characterized by the following: Their cellular outlines are indeterminable but their oval nuclei are large, about 7.5 microns, and each has a nucleolus. The nuclear membrane is distinct and encloses a clear karyolymph in which chromatic granules of varying sizes are irregularly distributed. The Sertoli cells are not found about the spermatophores after they enter the sperm duct. Medlen (1950) found the Sertoli cells in *Gambusia* before but not after the spermatophores pass into the lumen of the testicular canal.

The Release of Spermatophores.

The spermatophores pass along the branches of the sperm duct to the center of the testis where the two large ducts are fused and form a common, ciliated and tubular chamber, the so-called vas deferens (Pl. III, Figs. 8 & 10). The tissue about the wall of the caudal region of the sperm duct is thickened considerably, forming a genital papilla at its terminus. The duct opens at the apex of the genital papilla which, in turn,

projects into the uro-genital sinus. Just anterior to the genital papilla the sperm duct is surrounded by a smooth muscle sphincter which may be a part of the ejaculatory apparatus. Ventral to the sperm duct or genital sphincter and the genital papilla, a dense, fibrous connective tissue structure, in the shape of a shallow trough, lies for its entire length between the sperm duct and the terminal portion of the alimentary canal. This partition effectively separates the digestive system from the urinary and genital systems and extends to separate the anus from the urino-genital apertures (Pl. IV, Fig. 12).

Interrelationships Among Age, Length, Maturation of Testis and Gonopodium.

A summary of the various stages in the differentiation of the testis is given in Table I. In Table II the characteristic features of various developmental stages of the gonopodium are listed. The age, length, depth and the developmental stages of the gonopodia and testes of the fish of brood 260 are given in Table III and those of brood 263 are given in Table IV A, B.

Three product moment correlations, expressed as the coefficient r, were performed in order to determine the relationships, if any, among the values for the age, the standard length, the developmental stage of the testis and the developmental stage of the gonopodium of the male platyfish in 40 fish of brood 263. The measurement and developmental stages are given in Table IV; see also Text-figs. 1, 2 and 3.

Each value for r was transformed to the normally distributed coefficient z in order to increase the reliability of the calculations.

The first correlation coefficient was derived from a comparison of the ages of the fishes with their standard lengths. It is quite high, $r = +0.90$ and $z = +1.47 \pm 0.16$. The result obtained is what would be expected if there were no significant errors of sampling nor important variations in the environmental conditions under which the fish were kept. Under uniform conditions and similar genetic constitutions it may be said that the standard length of a fish increases in proportion to its advancing age.

The second series of calculations was made to obtain a comparison of the standard length of the platyfish with the develop-

TABLE 1.

Stages in the Differentiation of the Testis of *Platypoecilus maculatus*.

Stage 1: Primordial Germ Cells.	a. Two small, separated gonad primordia. *b. Gonad primordia largely composed of stroma. *c. Primordial germ cells discrete or in very small groups at the periphery of testis. d. No ducts present.
Stage 2: Spermatogonial Acini Few.	a. Testis distinctly bipartite. *b. In each primordium some stroma cells form one large duct. c. Much stroma still present. *d. Germ cells (spermatogonia), at the periphery of gonad primordia; not more than eight cells in small acini.
Stage 3: Spermatogonial Acini Numerous.	a. Testis somewhat less bipartite. *b. Appearance of many branching ducts. c. Stroma is greatly reduced. *d. Large number of germ cells (spermatogonia) in each of many acini.
Stage 4: Primary Spermatocytes.	a. Testis somewhat less bipartite. b. Ducts somewhat larger in size. c. Little stroma present. *d. Acini contain primary spermatocytes; comparatively few acini contain spermatogonia.
Stage 5: Secondary Spermatocytes.	a. Testis enlarged greatly. b. Sperm ducts more advanced. c. Little stroma present. *d. Cords of primary and secondary spermatocyte acini present; spermatids appear.
Stage 6: Spermatophores.	a. Fused gonad barely shows bipartite origin. b. Sperm ducts completely differentiated. c. Cords of acini numerous. *d. Spermatophores present in sperm ducts and tubules.

* Indicates the key characters.

TABLE 2.

Stages in the Development and Differentiation of the Gonopodium of *Platypoecilus maculatus*.

STAGE 1: Juvenile Phase.	Unmodified anal fin composed of nine pairs of fin rays.
STAGE 2: Preliminary Phase.	Thickening of the third ray of the anal fin. The beginning of the bifurcation of rays three and four.
STAGE 3: Growth Phase.	Elongation of rays three, four and five. Nine to ten segments in the third ray.
STAGE 4: Differentiation.	a. The appearance of a subdermal thickening. b. The appearance of the proximal serrae. c. The appearance of the distal serrae. d. The appearance of a blade over the hook.
STAGE 5: Completion.	Tip of gonopodium fully segmented and differentiated.

mental stage of its testis. The correlation coefficient is high, $r = +0.94$ and $z = +1.74 \pm 0.16$.

The third correlation coefficient was evaluated in order to determine the degree of association between the paired values for the standard lengths of the fish and the developmental growth and differentiating stages of their gonopodia. The result shows a high correlation, $r = +0.87$ and $z = +1.33 \pm 0.16$.

Summing up these correlations, it may be said that as the age of the platyfish increases, its standard length increases proportionately. As the standard length increases, the developmental stages of the testis advance. At the same time as its standard length increases, its anal fin develops in a successive series of stages to form the gonopodium (Text-fig. 1).

Brood 263 consisted of males of two genotypes. About half had the sex-linked gene *Sp* for macromelanophores irregularly arranged on the body, while the others had an allele *N* for large black pigment cells arranged in the form of a black band. A comparison was made between the two kinds with respect to their rate of growth (that is, age with reference to standard length) and to the rate of development of their testes and gonopodia. No significant differences between the two groups were found. The data are given in Table IV C.

Discussion.

The maleness of 71 platyfish out of 262 males of two broods was determined, in part, by histological studies of the gonads of the youngest members. Only testicular elements were found in all except the embryos and in the latter the gonads were indifferent. No ovarian structures were ever found. This is in contrast to the situation in some sword-

TABLE 3.

Measurements of *Platypoecilus maculatus* of Culture 260 Arranged in Regard to the Developmental Stage of Their Gonads.

Animal	Standard Length (mm.)	Standard Depth (mm.)	Body Index[1]	Stage of Gonopodium Development[2]	Stage of Testicular Development[3]
260- 1	10.8	3.5	3.1	1	1
260- 3	12.9	3.2	3.2	1	1
260- 4	12.9	4.5	2.9	2	1
260- 5	11.5	3.8	3.0	1	2
260- 6	11.6	3.8	3.1	1	2
260- 2	11.9	3.9	3.1	1	2
260- 7	18.2	6.0	3.0	2	3
260- 8	19.0	6.3	3.0	2	3
260-17	21.0	8.0	2.6	2	3
260- 9	18.7	6.7	2.8	2	4
260-10	21.9	7.9	2.8	3	5
260-18	22.0	8.0	2.7	3	5
260-15	25.1	9.5	2.7	5	5
260-12	29.0	11.0	2.6	5	5
260-16	22.5	8.5	2.6	3	6
260-14	23.0	8.0	2.9	4	6
260-11	24.0	8.5	2.8	5	6
260-13	26.8	9.5	2.8	5	6

Number of animals = 18.

[1] Value of standard length divided by the value of the standard depth.
[2] Regrouped from Table II.
[3] Regrouped from Table I.

TABLE 4A.

Measurements of Two Genetically Different Groups of Male Siblings of *Platypoecilus maculatus* of Culture 263 Arranged in Regard to the Developmental Stage of Their Gonads.

A. Platyfish with Macromelanophores forming Black Bands (*N*).

Animal	Standard Length (mm.)	Standard Depth (mm.)	Body Index[1]	Stage of Gonopodium Development[2]	Stage of Testis Development[3]	Age (Days)
263-23	5.5	1.3	4.2	*	*	1
263-24	5.5	1.3	4.2	*	*	1
263-22	6.0	1.3	4.6	*	*	1
263-37	8.7	2.7	3.2	1	1	28
263-35	9.5	3.0	3.2	1	1	28
263-36	11.2	3.8	2.9	1	1	28
263-18	9.5	3.0	3.2	1	2	50
263-10	12.9	4.0	3.2	1	2	73
263-34	14.3	4.5	3.2	1	2	137
263- 5	13.8	4.5	3.1	2	3	109
263-17	12.0	3.5	3.4	2	3	50
263-16	13.5	4.5	3.0	2	3	50
263-11	14.5	4.8	3.0	2	3	73
263-12	14.5	4.9	3.0	2	3	73
263- 6	14.0	4.7	3.0	2	4	109
263-32	16.7	5.2	3.2	2	4	137
263- 4	16.5	5.8	2.9	3	5	109
263-33	18.0	5.4	2.8	4	6	131
263-27	18.0	7.6	2.8	5	6	131
263-28	18.5	6.5	2.8	5	6	131

Number of animals = 20.

[1] Value of standard length divided by the value of the standard depth.
[2] Determined by Table II.
[3] Determined by Table I.
* These sexually undifferentiated animals were not included in the correlation studies.

TABLE 4B.

Measurements of Two Genetically Different Groups of Male Siblings of *Platypoecilus maculatus* of Culture 263 Arranged in Regard to the Developmental Stage of Their Gonads.

B. Platyfish with the Macromelanophores Scattered (*Sp*).

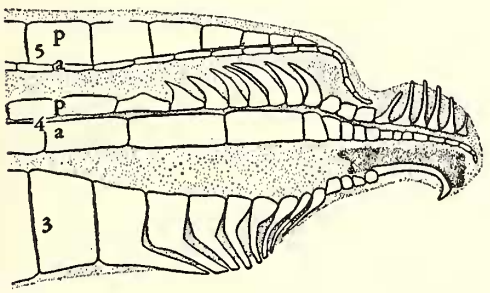
Animal	Standard Length (mm.)	Standard Depth (mm.)	Body Index[1]	Stage of Gonopodium Development[2]	Stage of Testis Development[3]	Age (Days)
263-20	5.5	1.3	4.2	*	*	1
263-19	6.0	1.3	4.6	*	*	1
263-21	6.0	1.5	4.0	*	*	1
263-40	8.8	2.8	3.1	1	1	28
263-38	9.3	3.1	3.0	1	1	28
263-39	9.8	3.0	3.3	1	1	28
263- 8	11.8	3.8	3.1	1	2	73
263- 9	12.0	3.2	3.7	1	2	73
263- 7	13.2	4.2	3.1	1	2	73
263-13	10.5	3.5	3.0	1	3	50
263-14	11.3	4.8	3.0	1	3	50
263- 1	13.5	4.5	3.0	2	4	109
263-15	12.0	4.5	2.7	2	5	50
263- 2	15.5	5.5	2.8	3	5	109
263-29	15.5	4.8	3.0	3	5	137
263- 3	16.0	5.8	2.8	3	5	109
263-26	16.7	5.8	2.8	4	6	131
263-31	17.0	5.4	3.1	4	6	137
263-30	15.1	5.0	3.0	5	6	131
263-25	17.0	6.0	2.8	5	6	131

Number of animals = 20.

[1] Value of standard length divided by the value of the standard depth.
[2] Determined by Table II.
[3] Determined by Table I.
* These sexually undifferentiated animals were not included in the correlation studies.

tails, *Xiphophorus hellerii*, and in some guppies, *Lebistes reticulatus*. According to Freiss (1933), Regnier (1938) and Dildine (1936), some males of these species when very young have ovarian elements which later degenerate and a normal testis develops.

The maleness of the 256 playfish of two broods was apparently determined genetically. This may be explained by the peculiar genetic constitutions of the parents of the two broods. The female parents had two X chromosomes, or XX, and the male parents had two Y chromosomes, or YY. All their offspring had XY and were male. The two all-male broods analyzed here are only a few of many which have been observed.



TEXT-FIG. 1. Fully differentiated distal tip of the gonopodium of *Platypoecilus maculatus*. The rays are numbered 3, 4 and 5. The letters *a* and *p* refer to the anterior and posterior halves of rays 4 and 5. The biramous parts of ray 3 have fused to produce the strongest element in the gonopodium. At the extreme distal tip, the elements of 4*p* are distal serrae; those of 4*a* form the ramus. The curved terminal element of ray 3 is the hook, the main element of the holdfast mechanism of the gonopodium. Above the hook is the blade which is shown in darker stippling. From Gordon & Rosen (1951).

From studies of maturation of the male platyfish, the following is our interpretation of the method of transport and ejaculation of the spermatophores. When first formed they lie in the germinal portion of the testis. They contain spermatozoa which are arranged peripherally with their heads surrounded by Sertoli cells. The spermatophore is encapsulated by a thin membrane derived from stroma cells. This confirms Wolf's observations, and a similar arrangement was described in the guppy by Goodrich, Dee, Flynn & Mercer (1934). Upon entering the sperm tubules the spermatophores are conveyed by the ciliary action of epithelial cells to the sperm duct terminus. Here they await ejaculation, which is effected when the genital sphincter is opened. The spermatophores then pass the terminal opening of the sperm duct at the apex of the genital papilla to the exterior.

TABLE 4C.

Comparison of Morphological Measurements and Developmental Features in Two Genetically Different Groups of Male Siblings of Brood 263 of *Platypoecilus maculatus*, Each Group Consisting of 20 Members[1].

Character:	χ^2	n
Standard Length	7.5	3
Depth	3.4	2
Testicular Development	3.2	2
Gonopodial Development	5.0	2

[1] These calculations were made from comparison of the data in Tables IV A and B.

The transformation of the male platyfish's anal fin into the gonopodium has been described in detail by Grobstein (1940). In the present study the various developmental stages of the gonopodium follow in sequence the developmental stages of the testis. These, in turn, follow directly with increasing age and size of the individual. Similar direct correlations have been noted in *Xiphophorus hellerii* by Van Oordt (1925), in *Gambusia* by Dulzetto (1933) and Turner (1941), and in *Lebistes* by Samokhvalova (1933).

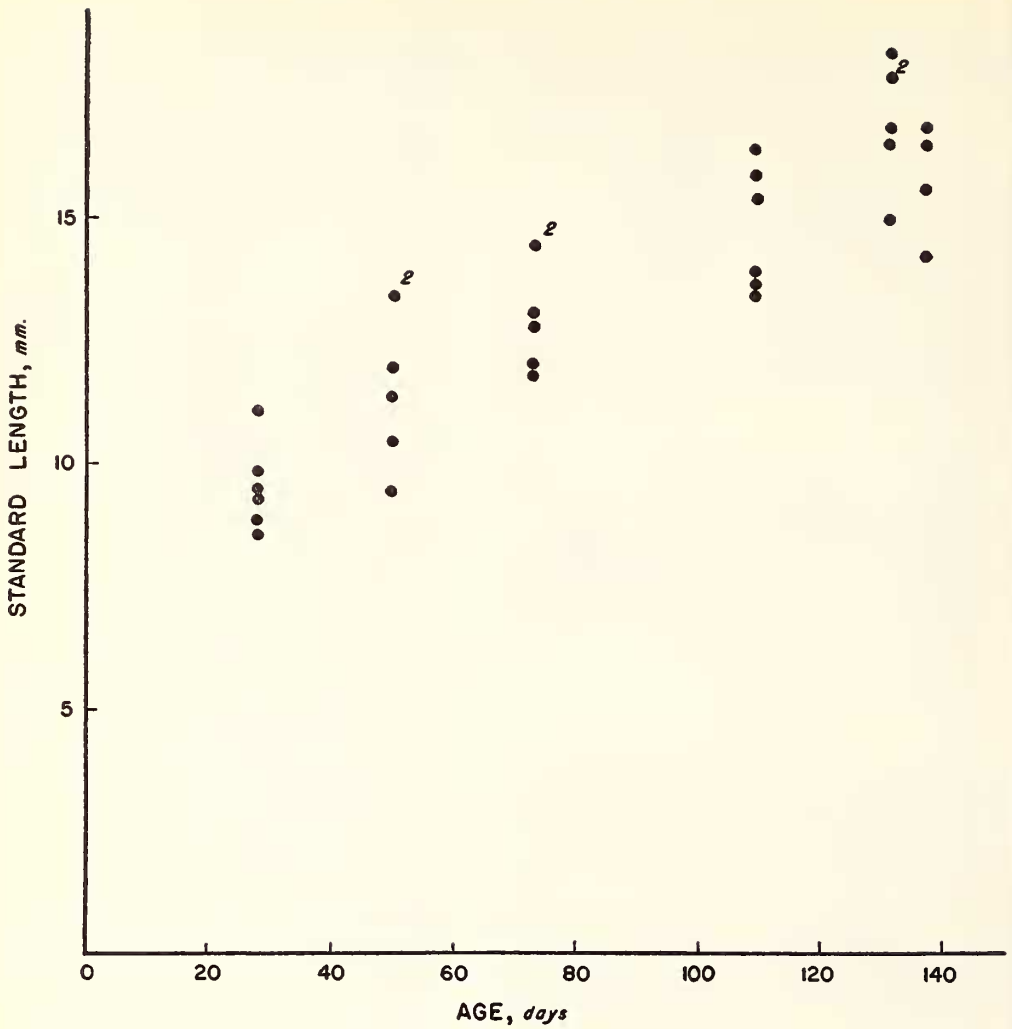
According to Witschi's theory of "corticomedullary inductors," (1939-1942), sex differentiation in the lower vertebrates is the resultant of an antagonistic action between the female or cortical and the male or medullary components of the gonad. Sex is decided by one component becoming dominant over the other. This is accomplished by a process of inhibition, one component apparently suppressing the other through the action of specific substances, cortexin and medullarin, assumed to be produced by cortex and medulla respectively. Which of the opposing components dominates is determined by their relative strength or power. Cortex "weaker" than medulla points to maleness; cortex "stronger" than medulla points to femaleness.

These concepts were derived from studies of the developmental stages in gonadal differentiation in amphibia and from experiments with them designed to evaluate the external factors, such as temperature, hormones, etc., which are capable of overriding the genetic sex constitution of the individual.

The lower vertebrates represent an extremely diverse group of animals and this may also be said of the teleosts alone. We doubt if the regional areas of the platyfish gonad can be compared satisfactorily on a morphological level, with those of *Rana*, for example. If the comparison is made, for whatever it is worth, the peripheral region of the platyfish gonad seems to be associated with the most important cells of the testis and the central core with components of the ovary. This is just opposite to the conditions in the frog. The effects of hormones on the teleost gonad are discussed in the accompanying paper by Gordon & Aronowitz (1951).

SUMMARY AND CONCLUSIONS.

1. A histological examination of the gonads of very young members of *Platypoecilus maculatus* in two broods has revealed that



TEXT-FIG. 2. Scatter diagram of the relationship of age to standard length of the fishes of brood 263. N = 40; $r = +0.90$.

all the members are male. No ovaries or ovary-like gonads were found. This confirmed the observation that all the members of the two broods numbering 262 individuals are male, none are female.

2. The genetic mechanism for sex determination responsible for the production of the two all-male broods was known. The female platyfish had two X chromosomes (XX), and the male platyfish had two Y chromosomes (YY); all their offspring possess an identical combination of sex chromosomes, XY, which is characteristic of the male. This genetic theory was confirmed by the histological results obtained.

3. During morphogenesis of the testis the following processes were found (they are also summarized in Table 1):

a. Fusion of the two testicular primordia.
b. Increase in size of the testis.
c. Spermatogenesis.

4. Spermatophores accumulate in the sperm duct. Upon relaxation of the genital sphincter, the spermatophores pass through the terminal opening of the sperm duct over the genital papilla to the exterior and to the gonopodium.

5. As the platyfish grows older, it increases in length. At the same time, as the testis matures, the anal fin is transformed into the gonopodium.

6. No difference in the rates of development of the testis and gonopodium was found in the Black-banded (*N* gene) and Spotted (*Sp* gene) siblings of the platyfish.

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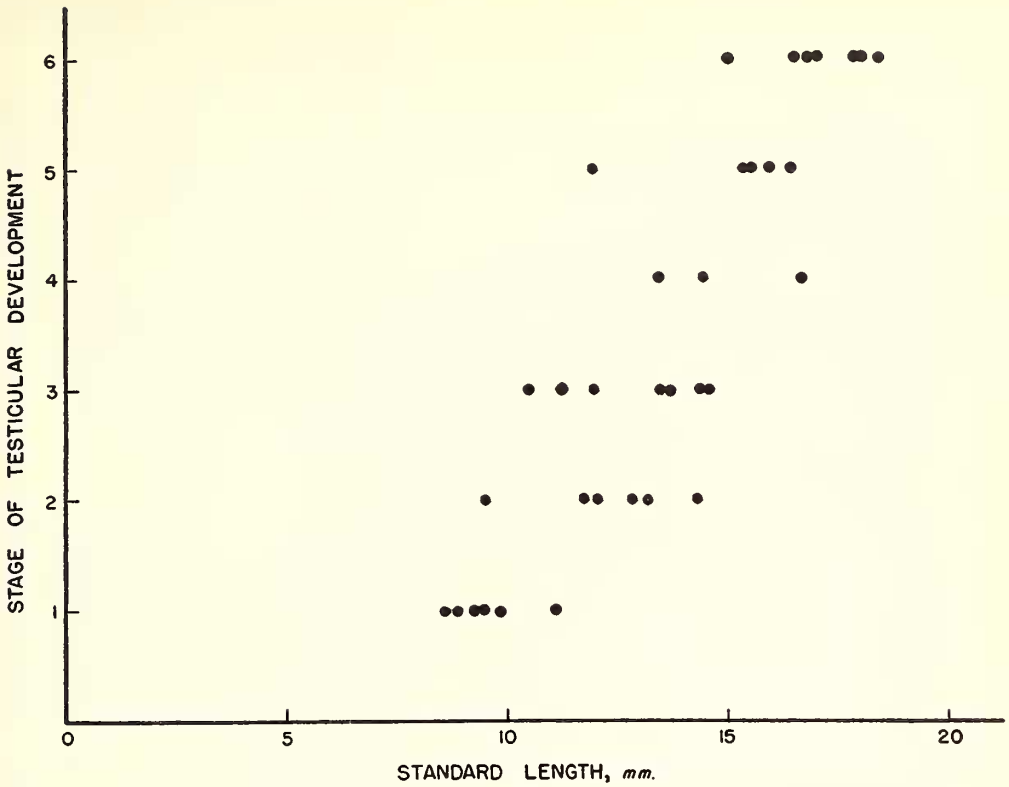

TEXT-FIG. 3. Scatter diagram of the relationship of the standard length of a fish to its stage of testicular development in the fishes of brood 263. $N = 40$; $r = +0.94$.

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EXPLANATION OF THE PLATES.

PLATE I.

Fig. 1. *Sexually Indifferent Gonad.* The small gonad primordia contain discrete primordial germ cells randomly distributed. There is no visible cortex or medulla. Embryo 263-B. Transverse section. Iron hematoxylin. Approximately 1500×. **PGC**, primordial germ cell.

Fig. 2. *Testicular Stage One.* The paired testis is composed of germ cells which occur discretely and in small groups at the periphery of the gonad. The bulk of gonad is composed of stroma cells. Animal 260-3. Transverse section. Harris' hematoxylin. Approximately 1200×. **PGC**, primordial germ cells; **S**, stroma cell.

Fig. 3. *Testicular Stage Two.* The small spermatogonial acini are critically located. In each testis the stroma cells of the medullary region are transformed into a large central sperm duct. Animal 260-5. Transverse section. Harris' hematoxylin. Approximately 450×. **D**, sperm duct; **S**, stroma cell; **SG**, spermatogonial cell.

Fig. 4. *Testicular Stage Three.* The peripheral region is thick and forms a distinct outer zone, which at this level of development is composed mainly of large spermatogonial acini. The branched sperm ducts are the major components of the medullary region. Animal 263-5. Transverse section. Harris' hematoxylin. Approximately 400×. **M**, mesorchium; **SG**, spermatogonial cell; **T**, sperm duct tubule.

PLATE II.

Fig. 5. *Testicular Stage Four.* The cortical region is considerably thicker. Primary spermatocytes predominate. Animal 263-6. Transverse section. Heidenhain's modification of Masson's trichrome stain. Approximately 300×. **M**, mesorchium; **P**, peritoneal membrane; **PSP**, primary spermatocyte acinus; **SG**, spermatogonial cell; **T**, sperm duct tubule.

Fig. 6. *Testicular Stage Five.* The secondary spermatocyte acini appear at this stage of testicular development; they are for the most part found near the center of the testis. For the first time the linear arrangement of the acini which form the cords may be seen. Animal 263-3. Transverse section. Harris' hematoxylin. Approximately 90×. **D**, sperm duct; **PSP**, primary spermatocyte acinus; **SSP**, secondary spermatocyte acinus.

Fig. 7. *Testicular Stage Five.* Note the growth and incomplete fusion of the paired testis. The cords of acini are pronounced. In the medullary region a sperm duct tubule opens into the left sperm duct. Animal 260-12. Transverse section. Harris' hematoxylin. Approximately 90×. **BV**, blood vessel; **CH**, cord of acini; **D**, sperm duct; **PSP**, primary spermatocyte acinus; **SSP**, secondary spermatocyte acinus; **T**, sperm duct tubule.

PLATE III.

Fig. 8. *Testicular Stage Six.* The bipartite origin of the testis may be discerned dorsally. Spermatophores are present both in the cortex and in the sperm duct network. The union of the paired sperm ducts to form the vas deferens may be seen. Animal 260-16. Transverse section. Harris' hematoxylin. Approximately 80×. **D**, sperm duct; **SP**, spermatophore; **T**, sperm duct tubule; **VD**, vas deferens.

Fig. 9. *The Later Stages of Spermatogenesis.* Note the indistinct cytoplasmic limits of the primary spermatocytes, the secondary spermatocytes and the spermatids. The nuclei of the primary spermatocytes are much larger than those of the secondary spermatocytes. Note the mature and immature spermatophores which are recognizable by the peripheral aggregation of the spermatozoa. Animal 263-30. Longitudinal section. Heidenhain's modification of Masson's trichrome stain. Approximately 450×. **PSP**, primary spermatocyte acinus; **SC**, Sertoli cell; **SP**, spermatophore; **SSP**, secondary spermatocyte acinus; **ST**, spermatid acinus; **T**, sperm duct tubule.

Fig. 10. *Ciliated Cuboidal Epithelium Lining the Sperm Ducts.* Note the sperm duct fluid which stains with aniline blue. Animal 263-30. Longitudinal section. Heidenhain's modification of Masson's trichrome stain. Approximately 450×. **C**, cilia; **DE**, duct epithelium; **E**, erythrocyte; **F**, sperm duct fluid; **St**, spermatid acinus.

Plate IV.

Fig. 11. *Terminal Portion of the Immature Male Reproductive System.* The testis is in Stage Two. The cephalic portion of the sperm duct leading from one testis is branched. The remaining portion of the duct is unmodified. Animal 263-9. Longitudinal section. Iron hematoxylin. Approximately 120×. **BL**, urinary bladder; **D**, sperm duct; **I**, intestine; **TS**, testis; **U**, ureter. (In this Figure and in Figure 12 the anterior-posterior axis runs from left to right).

Fig. 12. *Terminal Portion of the Mature Male Reproductive System.* Note the increase in size and the modifications of the genital system, compared with Fig. 11. The testis and sperm ducts have increased in complexity. A genital sphincter is found around a portion of the sperm duct at the base of the genital papilla. The genital opening is at the apex of the genital papilla. A partition separates the genital system from the digestive system and forms a definite uro-genital sinus. Animal 263-28. Longitudinal section. Heidenhain's modification of Masson's trichrome stain. Approximately 120×. **A**, anus; **BL**, urinary bladder; **GP**, genital papilla; **GS**, genital sphincter; **P**, partition; **UGS**, uro-genital sinus; **VD**, vas deferens.